Understanding the Electrophysiological Changes in Microgravity and Radiation Exposed Bacteria using Dielectrophoresis and Impedance Methods

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Introduction

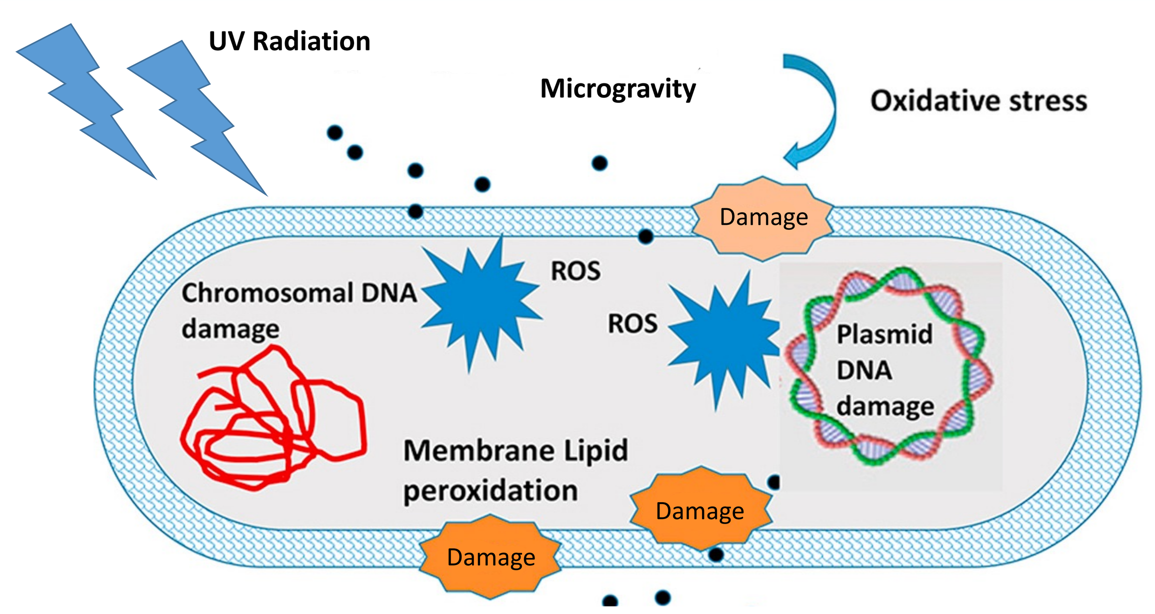


Fig 1. Damage Caused by Increased ROS Production from External Stressors

Microgravity and radiation are extreme environments in space that living organisms will need to face during long-term space travel. Therefore, it is important to thoroughly research the effects of these extreme environments on living organism. Microgravity and radiation has shown to cause both up and down regulation of certain genes,[1] increased and decreased virulence,[2,3] and even impaired or improved biofilm formation.[4] In other words, depending on the bacterial species, the organism can react to microgravity differently, further justifying the need for experimentation on different bacterial species; the effects of microgravity on specific bacterial species needs to be determined experimentally. A major byproduct of microgravity and radiation is the increased production of Reactive Oxygen Species (ROS), which have been shown to cause damage to nucleic acids, proteins, and lipids.[5] ROS species have also been found in high concentration in the plasma membrane of bacteria, which is the site of cellular respiration in prokaryotic cells. Figure 1 represents the production of ROS species in bacteria. Moreover, the increase in ROS production can damage aspects of the cell membrane, causing changes in electrochemical properties of the cell. Thus, the given project seeks to look at radiation and microgravity exposed E. Coli samples, and observe the change in conductivity and permittivity of the cell membrane. By using methods such as dielectrophoresis, fluorescent microscopy, and impedance methods, insight into the health, geometry, growth, differentiation, function, physiological state, and the death of the biological cells, i.e., phenotype and genotype, can be measured.

Objectives

- **To understand the electrophysiological properties of bacteria (*E. coli)* when exposed to radiation, microgravity, and a combination of both conditions.**
- **Identify the relationship between cell conductivity, polarizability, and cell-membrane functionality.**
- **Measure changes in death rate, cell membrane morphology, and gene regulation of microgravity exposed *E. coli* cells**

Dielectrophoresis and Impedance

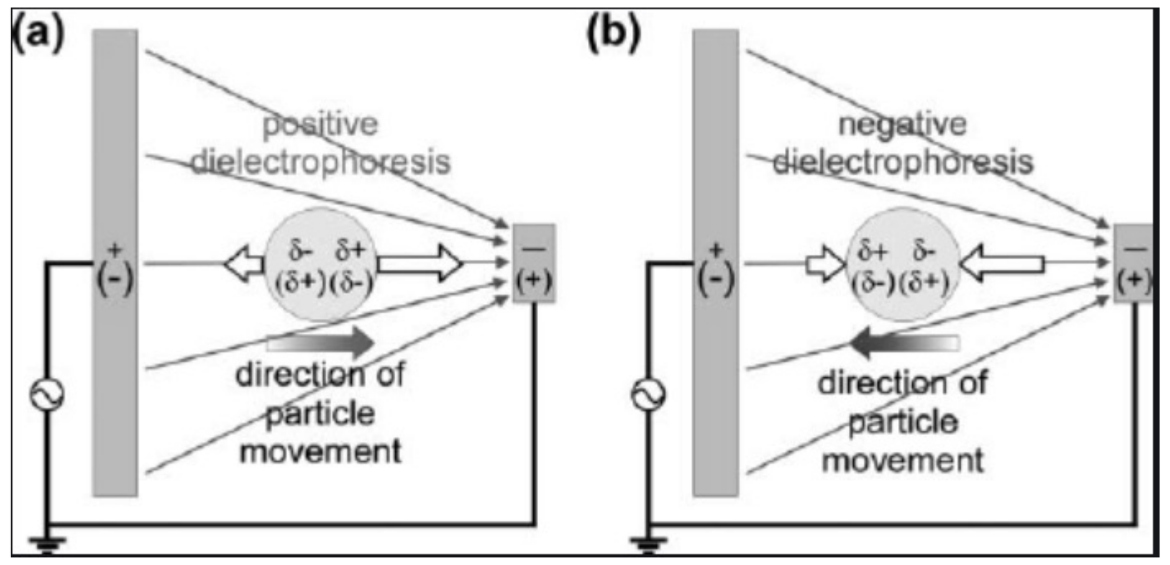


Fig 2. Explanation of the Dielectrophoretic Phenomenon **(a)** Positive Dielectrophoresis **(b)** Negative Dielectrophoresis

Dielectrophoresis (DEP) is a phenomenon that describes when a polarizable bioparticle is exposed to a non-uniform electric field, it moves in the direction of the high electrical field depending on the conductivity and permittivity of the particles and medium. The non-uniform electric field is produced from the wither size difference between the two electrodes, as shown in figure 1, or by manipulating the distance between the 2 electrodes. This phenomenon helps to manipulate the motion of the particles by creating a polarizable gradient between them in the suspending medium, causing positive Dielelctrophoresis (p-DEP) as shown in figure 1a and negative dielectrophoresis (n-DEP) as shown in figure 1b.

Impedance is the opposition of Alternating Current (AC) by the combined effect of resistance and reactance in a circuit. "Impedance Biology" utilizes electrical impedance, represented as |Z| in Equation 1, to be used for monitoring, detection, and analysis of microorganisms

$$|Z| = \sqrt{R_s^2 + \left(\frac{1}{2\pi f C_{dl}}\right)^2}$$

Equation 1. Measurement of Electrical Impedance

Microgravity and Radiation

3D Printed Clinostat with Radiation Survey Meter

A 3D printed clinostat containing exposure to radioactive cobalt-60 and cesium-137 is used to simulate space conditions. The process begins with a standard *E. coli* overnight culture. The culture is then placed in individual tubes and rotated in the clinostat for varying amounts of time.

The clinostat utilizes a "slow rotation" to cause constant suspension and freefall within the tubes, in turn, simulating microgravity. After desired concentration is obtained, DEP, impedance studies, and fluorescent tagging is used

Experimental Results

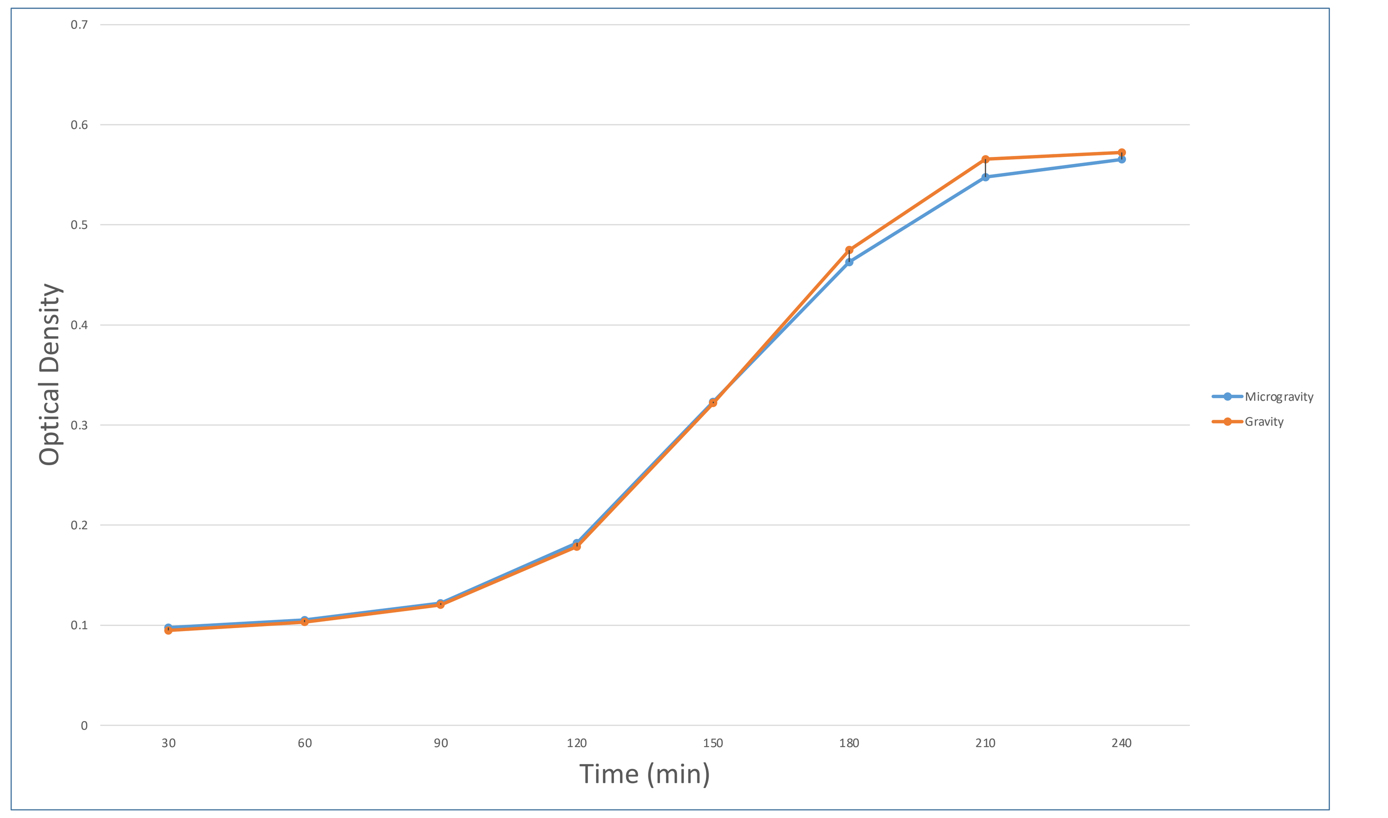
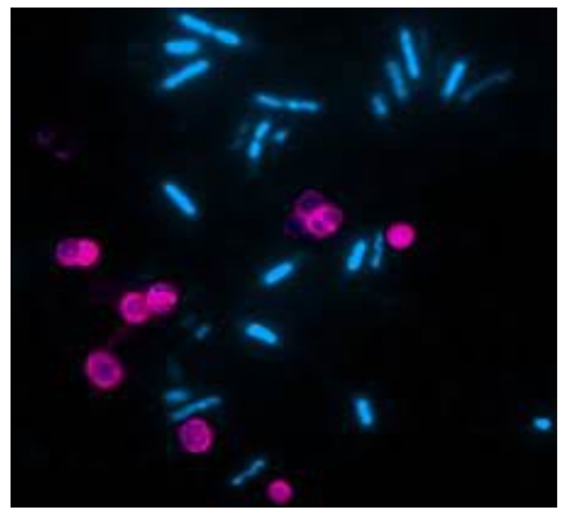


Fig 3. Comparison of K-12 *E. coli* Growth Cycle in Microgravity and Gravity Conditions

Fig 4. Theoretical Representation of fluorescent tagged bacteria using *E. coli* serotype O/K Polyclonal Antibody



Discussion and Conclusions

Based on literature, E. coli exposed to microgravity conditions has shown significant alterations to gene expression and the growth cycle. Figure 3 represents the beginning of a K-12 *E. Coli* growth curve. It is theorized the "death phase" of the growth curve will be substantial in those exposed to radiation and microgravity conditions, due to an increase in ROS production and changes in gene expression necessary to cell survival. Figure 4 represents a theoretical/expected image of the fluorescent tagged E. coli.

Future Outlook

The future outlook of this project includes the utilization of impedance methods and dielectrophoresis to measure the changes in cell conductivity, polarizability, cell membrane functionality, and more. Future experiments include the use of a Scanning Electron Microscope (SEM) topography, to get detailed images of the cell membrane of the microgravity and radiation exposed bacteria. The project also plans to use such methods to further understand the changes at a cellular level in space conditions.

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